Egyptian Journal of Biology, 1999, Vol. 1, pp 72-81
© Printed in Egypt. El Harmeen Press, Cairo.

Egyptian British Biological Society
(EBB Soc)

Electrophysiological effects of the solitary bee "*Anthophora pauperata*" venom on different types of muscles

Miran Rakha*, Aida Hussein, Zohour Nabil and Samy Zalat

Department of Zoology, Faculty of Science, Suez Canal University, Ismailia, Egypt

ABSTRACT

Effects of the crude venom of the solitary bee *(Anthophora pauperata)* on cardiac, skeletal and smooth muscles were studied to reveal the mechanism of action of this venom. The main toxic effects on the ECG of isolated toads' hearts noticed after perfusion with different concentrations of this venom were represented as a decrease in the heart rate (HR) accompanied by an elongation in the P-R interval. A gradual and progressive increase in the R wave amplitude was also noticed and verapamil abolished this effect. Several cases of cardiac disorders were recorded. These cases were represented as severe bradycardia, sinus arrhythmias and different degrees of A-V block. Moreover, S-T segment elevation or depression which indicate direct toxic effects on the heart as ischaemia and infarct were also noticed. Atropine and nicotine decreased these effects. Perfusion of the gastrocnemius muscle-sciatic nerve preparation of toads with 1 μg/ml venom solution decreased the mechanical contraction of the muscle without recovery. The electric activity of the rabbit's duodenum was recorded before and after the application of venom solution. The venom depressed the amplitude of muscle contraction. Pretreatment with atropine nearly abolished the effect of the venom.

KEYWORDS: solitary bee, *Anthophora,* venom, ECG, muscles.

INTRODUCTION

Eleven families of bees are recognized, five families (Stenotritidae, Oxaeidae, Melittidae, Ctenoplectridae and Fidellidae) contain only species with the solitary life styles. The majority of species in the families: Colletidae, Andrenidae, Halictidae, Anthophoridae and Megachilidae are also solitary, but all have some species which are, in the broadest sense, social. Family Apidae consists primarily of social species and embraces those with the most highly developed social behaviour (O'Toole & Raw 1991).

* Address for correspondence

The present knowledge of venoms of solitary bees is rather poor. Family Anthophoridae is a good example for studying the venoms of solitary bees, since it is a very large family and it is found in all parts of the world. This family is subdivided into three subfamilies, two of which are interesting regarding knowledge of their venoms: the Anthophorinae, including *Anthophora*, and Xylocopinae, including *Xylocopa*.

The venom structure of the solitary bee *Anthophora pauperata* was first described by Zalat *et al.* (1999), the data showed the complexity of structure of the venom of this species since it contains 18 bands, with possibility of occurrence of different enzymes and also a form of melittin molecule. In rat phrenic nerve-hemidiaphragm preparation, the venom of *Xylocopa violacea* caused a slow tonic contraction, which could not be maintained completely and was also slowly reversible. This type of slow contraction is completely similar to that caused by the venom of *Bombus terrestris* and by melittin from *Apis mellifera*, indicating that a melittin-like substance may be present in the venom (Piek 1986).

The whole venom of the solitary bee *(Anthophora pauperata)* was used in this study to reveal the mechanism of action of this venom in an attempt to eliminate its toxic effects on the cardiac, skeletal and smooth muscles.

MATERIALS AND METHODS

Venom collection

Venom was collected from the solitary bee *(Anthophora pauperata)*, a species which is endemic to the area of St Katherine, Sinai, Egypt. Pure venom was obtained by the method of Schmidt (1986). Frozen bees were thawed, sting apparatus was removed into a spot of distilled water. The venom reservoir was pinched off, removed from the rest of the sting apparatus and rinsed with distilled water. The venom was squeezed out of the reservoirs. The whole venom was dehydrated over silica gel for 3 days. Venom powder was dissolved in frog ringer solution for the experiments applied on toads while in sterile (0.9%) for the experiments applied on rabbits. The venom was stored at -20°C.

Animals

Adult male toads *(Bufo regularis)* 35-40 g body weight and male rabbits weighing about 0.5 kg were used in this investigation as an experimental model for the *in-vitro* study.

In-vitro experiments and doses

Cardiac muscle

Experiments on cardiac muscle were carried out on the isolated toad's heart preparations. Three groups each of 10 animals were used. They were directly perfused with 0.25, 0.5 and 1µg/ml, concentrations were chosen according to the preliminary tests. Electrocardiogram (ECG) was recorded directly from the surface of the heart before venom application to serve as self-control. After venom perfusion, signals were recorded each five minutes for half an hour. ECG was recorded by the multipen rectilinear recorder DBE, UK with paper speeds 2 & 10 mm/sec.

Skeletal muscle

Toad's gastrocnemius muscle-sciatic nerve preparations (n =10) were used to study the effect of solitary bee venom on the mechanical contraction of skeletal muscle. Muscles

were directly perfused with 1 µg/ ml of the venom solution. Activity of the skeletal muscle was recorded by 10500 ink kymograph, Bioscience, UK with paper speed 1mm/sec. Sciatic nerve was electrically stimulated with 1V, 1.4 m sec, 1 hz square pulse waves.

Smooth muscle

Rabbits were killed by a blow on the back of the neck, the abdomen was immediately opened and duodenum was excised and placed in a bowl of Tyrode solution. A mono-organ bath with an inner vessel 40 mm was used where gut preparation was mounted. Effect of the venom was studied by adding 1µg/ml venom solution to the bath. The activity of the muscle was recorded by means of T3 auxotonic transducer, FC 100 direct input coupler on the two channel curvilinear oscillograph MD2 Bioscience, UK with chart speed 0.25 mm/ sec.

Antagonists

Atropine sulphate was purchased from Memphis Co, Dorpharm and Chem, Ind. Cairo. A concentration of 4 µg/ ml ringer was used on heart while $5x10^{-6}$ M in saline was added into smooth muscle preparations. Nicotine with a large concentration 1% was used on the myocardium. It was brought from Merck- Schuchardt. Verapamil hydrochloride (40 mg), the calcium channel blocker, was used on the heart with a concentration of 5 µg/ ml. It was purchased from ADWIC pharmaceutical division, El Nasr Pharm. Chem. Co., Abu Zabal, Egypt. Flaxedil manufactured by Alex. Pharm. Co., Egypt, it was used on the skeletal muscle with a concentration of 3 µg/ ml ringer solution.

Statistical analysis

Responses of heart rate (HR) and the other electrocardiographic parameters (P-R interval, R-wave amplitude) to venom treatments were expressed as means ± standard errors. Differences in the heart rate (HR) and the other ECG parameters were analysed using student's paired t-test ($p < 0.05$) according to Snedecor & Cochran (1980). Differences between several concentrations in the same parameter were analysed by one-way analysis of variance (Anova-test) at $P < 0.05$ according to Snedecor & Cochran (1980).

RESULTS

Low concentration (0.25, 0.5 and 1µg/ml) of *Anthophora pauperata* venom solution induced changes as well as toxic effects to isolated toad's heart, these effects lasted 30 minutes, while higher concentration (more than 1µg/ml) stopped the heart shortly. These low concentration resulted in sever bradycardia, an increase in the P-R interval as well as increasing the R wave amplitude. The significant bradycardia began from the onset of venom application and lasted 30 minutes in the low and medium doses while lasted 20 minutes in the high dose (Table 1). The venom has lead to a significant increase in the P-R interval indicating decrease of conduction velocity till the end of the experiment (Table 2). The myocardiae contractility (R wave amplitude) was increased significantly after venom application as shown in Table (3).

Table 1: Effect of direct perfusion with the solitary bee *(Anthophora pauperata)* venom on the heart rate (HR) of isolated toad's heart. Values represent means ± se (n=10/group).
* Significantly different from zero value of the same dose, student's paired t-test ($p<0.05$).
* Non significant variance between concentration at the same time interval, Anova test ($p>0.05$).

Time (min)	*HR (B/min)*		
	0.25 µg/ml	**0.5 µg/ml**	**1 µg/ml**
0	74.8 ± 2.2	78.1 ± 2.8	77.0 ± 2.7
5	59.4* ± 2.9	64.4* ± 1.9	59.1* ± 4.3
10	54.8* ± 3.8	53.0* ± 3.5	49.3* ± 7.2
20	35.5* ± 4.4	32.0* ± 3.6	40.7* ± 10.2
30	29.6* ± 3.8	27.0* ± 4.2	

Table 2: Effect of direct perfusion with the solitary bee *(Anthophora pauperata)* venom on the P-R interval of isolated toad's heart. Values represent means ± se (n=10/group).
* Significantly different from zero value of the same dose, student's paired t-test ($p<0.05$).
* Non significant variance between concentration at the same interval, Anova test ($p>0.05$).

Time (min)	*P-R (m sec)*		
	0.25 µg/ml	**0.5 µg/ml**	**1 µg/ml**
0	310 ± 17.9	290 ± 10.0	290 ± 10.0
5	440* ± 16.3	460* ± 22.1	440* ± 16.3
10	500* ± 21.1	530* ± 30.0	466.7* ± 21.1
20	560* ± 26.7	575* ± 31.3	600* ± 57.7
30	562* ± 18.3	600* ± 37.8	

Table 3: Effect of direct perfusion with the solitary bee *(Anthophora pauperata)* venom on the R wave amplitude of isolated toad's heart. Values represent means ± se (n=10/group).
* Significantly different from zero value of the same dose, student's paired t-test ($p<0.05$).
* Non significant variance between concentration at the same time interval, Anova test ($p>0.05$).

Time (min)	*R (m v)*		
	0.25 µg/ml	**0.5 µg/ml**	**1 µg/ml**
0	1.18 ± 0.12	0.82 ± 0.08	0.94 ± 0.11
5	1.46* ± 0.10	1.06* ± 0.12	1.04* ± 0.14
10	1.38* ± 0.13	0.94* ± 0.19	0.87* ± 0.22
20	1.08* ± 0.16	0.95* ± 0.11	1.40* ± 0.31
30	1.03* ± 0.17	0.94* ± 0.09	

Table 4 shows incidences of ECG abnormalities as a result of the solitary bee (*Anthophora pauperata*) venom application. Plate (1) shows electrocardiograms taken from different isolated hearts treated with 0.25, 0.5 and 1µg/ml venom respectively. Several cases of cardiac disorders were noticed after perfusion with the venom. These cases were represented as different types of arrhythmias such as sinus arrhythmias and

different degrees of A-V block. S-T segment elevation or depressions were recorded which indicate direct toxic effects on the myocardium (Plate 2).

Table 4: Incidences of ECG abnormalities as a result of the solitary bee *(Anthophora pauperata)* venom application (n=10/group).

Cardiac Disorder	Number of incidences		
	0.25µg/ml	**0.5 µg/ml**	**1 µg/ml**
(A) Abnormal sinus rhythm:			
- Bradycardia:	10	10	10
- Sinus arrhythmias:	1	2	1
(B) Ectopic beats:			
- Atrial escape:	1	2	1
- Junctional escape:	5	3	3
- Premature atrial contraction:	-	-	1
(C) Atrioventricular block:			
- First degree:	10	10	10
- Second degree:	5	7	4
- Complete block:	3	5	5

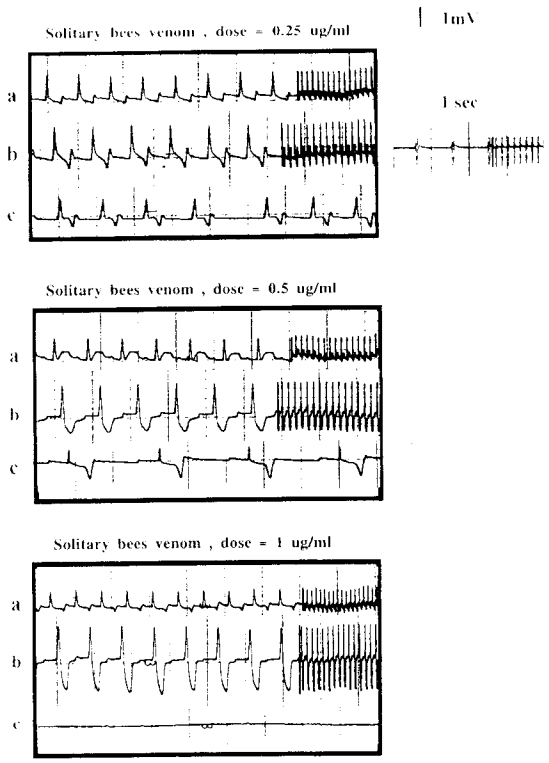

Plate 1: Electrocardiograms showing the effect of different concentrations of *Anthophora pauperata* venom on toad isolated perfused hearts. **a:** Before treatment **b:** 5 min after treatment. **c:** 30 min after treatment (in low and medium concentration) & 20 min after treatment (in the high concentration).

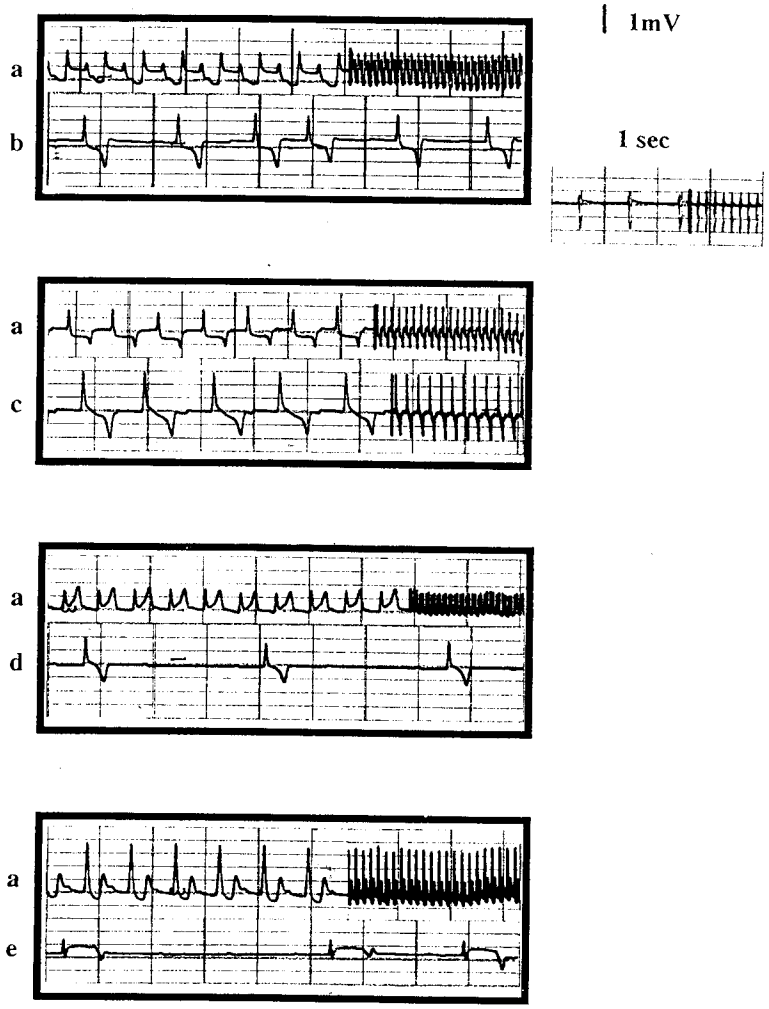

Plate 2: Examples of cardiac disorders in toad isolated hearts as a result of the solitary bee (*Anthophora pauperata*) venom application. **a:** Normal trace. **b:** Sinus arrhythmias. **c:** First degree block. **d:** Second degree block. **e:** Complete heart block

To study the mechanism of the venom action, atropine and nicotine were used as cholinergic blockers as well as verapamil as calcium channels blocker. Plate (3) represents the effect of adding atropine (1) or nicotine (2) or verapamil (3) into the toads' hearts perfused with the venom. As seen in cases (1) and (2), where the venom induced complete block, the heart started beating regularly when atropine or nicotine was added. The last case (3) in the same figure shows the effect of calcium channels blocker verapamil application on the positive inotropic effect of the venom where it has abolished the increased contractility noticed after 30 minutes following venom application.

The perfusion of the gastrocnemius muscle with 1 μg/ml venom solution decreased muscle contractions (Plate 4I). Blocking the nicotinic receptors with 3 μg/ml flaxedil before venom application almost sustained the normal contraction of the muscle. Adding the same concentration of the venom into the organ bath with the rabbit duodenum preparation induced a similar effect. The activity of the smooth muscles was impaired as seen in lowering the basal tonus of the muscle at the onset of venom application (Plate 4 II). Blocking the muscarinic receptors with atropine has abolished the noticed effect of the venom.

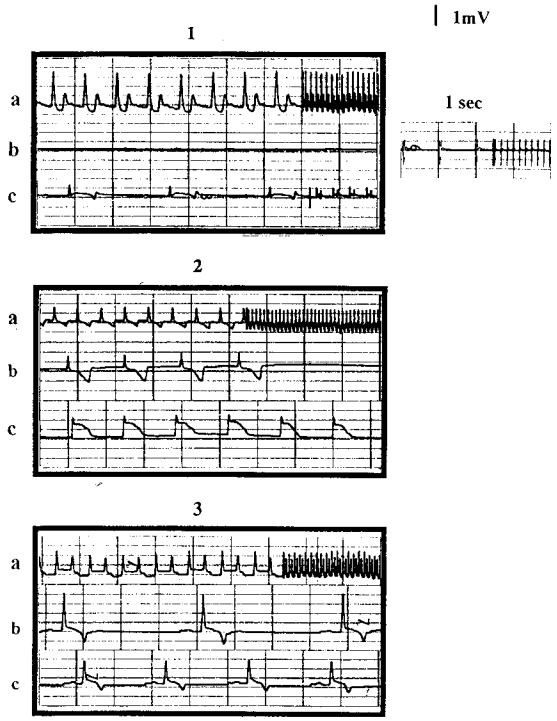

Plate 3: Effect of adding blockers into isolated toad hearts perfused with 0.25µg/ml of the solitary bee (*Anthophora pauperata*) venom solution. **1- Atropine.** **2-Nicotine.** **3- Verapamil.** a- Before venom application. b- 30 min after venom application. c- After adding the blocker.

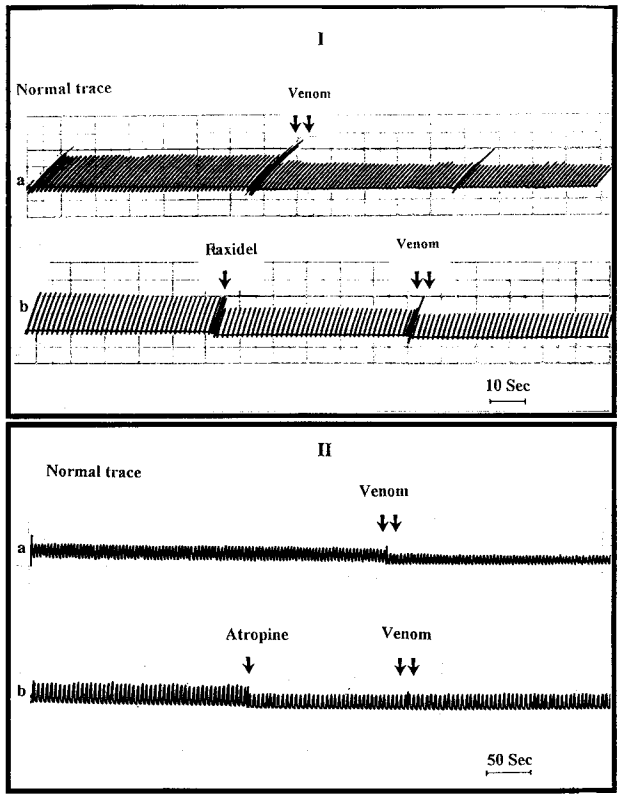

Plate 4: Effect of direct perfusion with 1µg/ml of *Anthophora pauperata*) venom solution on the: **I-** Mechanical activity of the toads gastrocnemius muscle. **II-** Electric activity of the smooth muscle of rabbits duodenum. **a:** Effect of the venom. **b:** pretreatment with blocker.

DISCUSSION

Our previous investigations studied the mechanism of action of the venoms of both the honey bee '*Apis mellifera*' (Nabil *et al.* 1998) and the bumble bee '*Bombus morrisoni*' (Hussein *et al.* 1999) on different types of muscles. The present investigation introduced some important results about the solitary bee *(Anthophora pauperata)* venom.

In this study, *in vitro* experiments were carried out on different muscle preparations. Isolated perfused organ preparations offer several advantages over experimentation on intact animals. Perfusion experiments lend themselves to a definite evaluation of the role of a particular organ or tissue in the disposition of endogenous or exogenous chemicals (Mehendale 1984).

The main toxic effects noticed in the present work were represented as severe bradycardia, sinus arrhythmias and different degrees of atrioventricular block indicating toxic effects on both sino-atrial and atrioventricular nodes of the heart. Moreover, S-T segment elevation or depression which indicate direct toxic effects on the heart as ischaemia and infarct were also noticed. These cardiovascular effects of *Anthophora pauperata* venom may be due to the synergism between its main components melittin and phosphalipase, since Marsh & Whaler (1980) noticed S-T segment depression, ventricular arrhythmias and ectopic beats on the ECG of isolated rat's heart as a result of honey bee venom administration and they attributed the major cardiovascular effects of honey bee venom to its two main components melittin and PLA_2. This also coincides with Ownby *et al.* (1997) who reported that melittin and contaminating PLA_2 in the melittin fraction from the honey bee venom may be acting synergetically to induce a stronger and more rapid myotoxic effects than occurs with either alone. Atropine abolished most of the above mentioned effects. This agrees with Korneva (1972) who reported that vagotomy eliminated the bradycardiac effects and reduced intensity of hypotensive action of the bee venom. Moreover, perfusion of the heart with large concentrations of nicotine also eliminated the noticed toxic effects. This leads to the suggestion that cardiac cholinergic receptors participate directly in the mechanism of action of *Anthophora pauperata* venom and that these effects may mediated through the peripheral cholinergic transmitter system.

Another obvious effect on the ECG parameters was the great increase in the R wave amplitude indicating an increase in the ventricular depolarization and consequently, contractile force of the ventricle. On the other hand, verapamil has completely abolished the noticed increase in the R wave amplitude. This proves that the positive inotropic effect of the venom might be due to the direct effect of the venom on the myocardium and consequently increasing the calcium influx. It is worth noting that PLA_2 leads to increasing the contractile force due to preventing uptake of released calcium in cytoplasm by receptors of endoplasmic reticulum (Tu & Baker 1989).

Direct perfusion of the skeletal muscle preparations with 1 µg/ml venom solution confirms the noticed toxic effects on the myocardium. Mechanical activity of the gastrocnemius muscle was decreased. This agrees with Piek *et al.* (1983), who noticed that in the presence of neostigmine, the venom of the bumble bee *(Bombus terrestris)* causes a decrease in the twitch amplitude of rat diaphragm and comparable effects on the rat diaphragm were found following treatment with the venoms of the solitary bee *(Xylocopa violacea)* and the honey bee *(Apis mellifera)*, as well as the melittin from the honey bee venom. Flaxedil prevented the venom from showing its

action, so it can be concluded that the venom action on the skeletal muscle was mediated through the nicotinic receptors. This coincides with the data of Hawgood *et al.* (1988), who attributed the decrease in frequency and onset of motor end plate (MEP) potential of the frog after perfusion with PLA_2 from the honey bee venom to inhibition of vesicular acetylcholine uptake.

Moreover, a depressor effect on the smooth muscle contraction by *Anthophora pauperata* venom was established in this study, since the amplitude of the muscle contraction was decreased. This effect may be mediated through the cholinergic peripheral neurotransmitter system, since atropine abolished this depressor effect.

Accordingly, it can be concluded that *Anthophora pauperata* venom may activate the parasympathetic system by releasing acetylcholine in the postganglionic nerve endings through axon reflex . Atropine antagonizes the depressor effects of *Anthophora pauperata* venom and tends to protect against arrhythmias. The noticed symptoms may reflect general neurotoxicity of an inhibitory nature involving the autonomic as well as neuromuscular system. In the meantime, direct effect of *Anthophora pauperata* venom on membrane integrity can also be concluded.

REFERENCES

Hawgood BJ, Smith I & Strong PN (1988) Early induction by crotoxin of biphasic frequency changes and giant miniature end plate potentials in frog muscle. *Br. J. Pharmacol.* 94: 765-772.

Hussein A, Nabil Z, Zalat S & Rakha M (1999) Effect of the bumble bee *'Bombus morrisoni'* venom on cardiac, skeletal and smooth muscle activity. *Egyptian Journal of Biology*. 1: 45-56.

Koneva NV (1972) Mechanism of change of blood pressure and frequency of heart beat caused by bee venom. *Uch. Zap. Gor'k. Gos. Univ.* 56.

Marsh NA & Whaler BC (1980) The effects of honey bee *(Apis mellifera* L.*)* venom and two of its constituents, melittin and phospholipase A_2, on the cardiovascular system of the rat. *Toxicon* 18: 427-435.

Mehendale HM (1984) Application of isolated organ techniques in toxicology. In Principles and methods of toxicology. Hayes AW (ed.). Raven Press, New York.

Nabil Z, Hussein A, Zalat S & Rakha M (1998) Mechanism of action of honey bee *(Apis mellifera* L.*)* venom on different types of muscles. *Human and Experimental Toxicology* 17: 185-190.

O'Toole C & Raw A (1991) Bees of the World. Blandford, London.

Ownby CL, Powell JR, Jiang MS & Fletcher JE (1997) Melittin and phospholipase A_2 from bee *(Apis mellifera)* venom cause necrosis of murine skeletal muscle *in vivo. Toxicon* 35: 67-80.

Piek T (1986) Venoms of bumble-bees and carpenter-bees. In Venoms of the Hymenoptera. (Pick T, ed.). Academic Press, London. pp.417-424.

Piek T, Veldsema-Currie RD, Spanjer W & Mantel P (1983) Acetylcholine and an unidentified muscle-contracting factor in the venom of the bumble bee, *Bomhus terrestris. L. Comp. Biochem. Physicol.* (75C): 351-356.

Schmidt JO (1986) Chemistry, pharmacology and chemical ecology of ant venoms. In venoms of the Hymenoptera. Piek, T.(ed.). Academic Press, London. pp. 425-508.

Snedecor GW & Cochran WG (1980) Statistical methods. 7th ed. Iowa State Univ. Press, U.S.A.

Tu AT & Baker BJ (1989) Effects of natural toxins on calcium channels and calcium pumps. *Comments Agric and Food Chemistry.* 2(1): 51-88.

Zalat S, Nabil Z, Hussein A & Rakha M (1999) Biochemical and haematological studies of some solitary and social bee venoms. *Egyptian Journal of Biology* 1: 57-71.

الملخص العربى

التأثيرات الإلكتروفسيولوجية لسم النحل الإنفرادى "*أنثوفورا بيبويراتا*" على أنواع مختلفة من العضلات

ميران رخا، عايدة حسين، زهور نبيل و سامي زلط

قسم علم الحيوان – كلية العلوم – جامعة قناة السويس – الإسماعيلية

تناولت الدراسة تأثير سم النحل الإنفرادى (*أنثوفورا بيبويراتا*) على كل من عضلة القلب والعضلات الهيكلية والملساء لمعرفة ميكانيكية تأثير هذا السم. تمثلت تأثيرات هذا السم على رسم القلب الكهربي للضفدع في حدوث نقص في معدل ضربات القلب مصحوبا بنقص سرعة التوصيل الأذيني البطيني. كذلك تسبب هذا السم في زيادة قوة إنقباض البطين وقد أزال الفيرابراميل هذا التأثير. ظهرت حالات عديدة تعكس حدوث خلل في عضلة القلب مثل بطء إيقاع القلب وعدم الإتساق الجيبي والسده القلبية. كذلك لوحظ حدوث إرتفاع أو إنخفاض في القطعة S -T والتي تعكس حدوث تأثيرات ذات سمية مباشرة على عضلة القلب مثل ذوى العضلة القلبية والإحتشاء القلبي وقد إستطاع كل من الأتروبين والنيكوتين تقليل هذه التأثيرات. بالنسبة للعضلة الساقية البطنية فإن غمرها بالسم قد أضعف من إنقباضها بشكل غير رجعى. هذا وقد منع إستخدام الفلاكسيديل هذا السم من إظهار تأثيره.

تم تسجيل النشاط الكهربي لمعي الأرنب قبل وبعد المعالجة بهذا السم. وقد تسبب هذا السم في نقص النشاط الكهربي للعضلات الملساء للأرنب ولم يظهر هذا التأثير في وجود الأتروبين.